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Past strong experiences determine acute cardiovascular autonomic responses to acoustic stress

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Abstract: **Background:** Stress is a major risk factor for cardiovascular (CV) disease. We hypothesized that past strong experiences might modulate acute CV autonomic responses to an unexpected acoustic stimulus.

Aim: The study's aim was to compare acute CV autonomic responses to acoustic stress between students with and without a past strong experience associated with the acoustic stimulus.

Materials and Methods: Twenty five healthy young volunteers — medical and non-medical students — were included in the study. CV hemodynamic parameters, heart rate (HR), and blood pressure (BP) variability were assessed for 10 min at rest and for 10 min after two different acoustic stimuli: a standard sound signal and a specific sound signal used during a practical anatomy exam (so-called “pins”).

Results: Both sounds stimulated the autonomic nervous system. The “pins” signal caused a stronger increase in HR in medical students (69 ± 10 vs. 73 ± 13 bpm, $p = 0.004$) when compared to non-medical students (69 ± 6 vs. 70 ± 10 , $p = 0.695$). Rises in diastolic BP, observed 15 seconds after sound stressors, were more pronounced after the “pins” sound than after the standard sound signal only in medical students (3.1% and 1.4% vs. 3% and 4.4%), which was also reflected by low-frequency diastolic BP variability (medical students: 6.2 ± 1.6 vs. 4.1 ± 0.8 ms², $p = 0.04$; non-medical students: 6.0 ± 4.3 vs. 4.1 ± 2.6 ms², $p = 0.06$).

Conclusions: The “pins” sound, which medical students remembered from their anatomy practical exam, provoked greater sympathetic activity in the medical student group than in their non-medical peers. Thus, past strong experiences modulate CV autonomic responses to acute acoustic stress.

Keywords: stress, students, autonomic system activity, heart rate variability, blood pressure variability.

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Introduction

Stress is one of the major risk factors for the development of debilitating cardiovascular disease. Therefore, the lowering of stress levels is an essential preventative measure in reducing cardiac mortality. Noise influences the cardiovascular system by causing increases in heart rate, cardiac output, and blood pressure. Such biochemical changes are multidimensional, with acoustic stress further causing changes in adrenaline, noradrenaline and corticosterone plasma levels.

Loud sounds cause stress which directly affects the autonomic nervous system (ANS) and cardiovascular hemodynamic parameters [1]. In previous studies of acoustic stimuli (music, noise), such stimuli caused responses in autonomic nervous system and cardiovascular system activity, e.g. increased heart rate (HR), diastolic blood pressure (DBP), low frequency (LF), and decreased high frequency (HF) parameters of heart rate variability (HRV) [2–6].

A non-invasive method for examining the autonomic innervation of the heart and the vegetative modulation of the sinus node is heart rate variability. Heart rate and blood pressure fluctuations are continuously changing under the control of the autonomic nervous system. This mechanism allows for the maintenance of homeostasis and ensures the appropriate functioning of the cardiovascular system. Measuring the frequency domain analysis of HRV and blood pressure variability (BPV) allows us to capture short and rapid changes in balance between sympathetic and parasympathetic part of ANS [5, 7–10].

Nonlinear methods have been applied to isolate the autonomic contribution to HRV modulation. HRV is the result of a complex regulatory system related to the electrical depolarization of cardiac cells, which is primarily regulated by the autonomic nervous system and the mechanical and functional properties of cardiac cells. Electrolytes acting on the refractory period of the action potential of cardiac cells also have an effect on this system. Nonlinear methods are useful tools in characterizing these properties of the cardiac regulatory system quantitatively from one of its measures, that is, the heart rate [11–13]. Certain pathological conditions, such as myocardial infarction, diabetes mellitus and aging are defined by the loss of dynamism in the heart rate regulatory system [13]. The most important role of nonlinear HRV indices is to improve clinicians' ability to identify patients at high risk of cardiovascular death. Nonlinear HRV indices provide unique information about a patient's cardiological status, accounting for its utility in a standard comprehensive cardiological diagnostic workup [13–15].

We hypothesized that sounds connected with stressful memories will provoke a more robust autonomic nervous system response and lead to stronger activation of the sympathetic system [12, 16]. In our study, we attempted to investigate the following questions: can exam-related sounds cause a stronger stress response in

students when compared with a similarly stressful standardized sound? Could the longer exam-related sound cause a more significant change in measured parameters than the shorter standardized sound?

The aims of our study were to assess the response to acute, short acoustic stimuli in ANS activity and cardiovascular system, and to determine the influence of individual experience and memories on the perception of stimuli and response. Additionally, we evaluated the time from stimulus to the onset of changes and the duration of response.

Materials and Methods

The study recruited 25 healthy volunteers: 15 students of the Jagiellonian University Faculty of Medicine and 10 students not linked with the Medical College who have never heard any anatomy exam signal. All participants had normal sinus rhythm in ECG, normal heart rate, and blood pressure within age-appropriate normal parameters. Anthropometric measures such as weight, height, BMI, and body surface area were obtained from each volunteer (Table 1). The study's exclusion criteria precluded the participation of volunteers with: diabetes mellitus, obesity (BMI >30), cardiovascular diseases (hypertension, coronary artery disease, valvular heart disease, cardiac arrhythmias), neurological diseases, or use of medication that may modulate cardiovascular or autonomic nervous system function.

To create conditions conducive to the calming of study participants, the study site was decorated in season-appropriate Christmas décor. All participants completed a questionnaire detailing their attitude to Christmas-time, and any participants with trauma associated with this season were excluded from the study. In addition, study participants completed a questionnaire assessing sources of stress, current level of stress (on the basis of symptoms), work under pressure, and stress coping mechanisms.

Table 1. Demographic characteristic of the participants. Legend: n — number of subjects, BMI — body mass index.

	All participants n = 25	Medical group n = 15	Non-medical group n = 10	p
Age [years]	21.96 ± 0.79	22.13 ± 0.74	21.7 ± 0.82	0.71
Gender [n]	16 males/9 females	10 males/5 females	6 males/4 females	
Height [cm]	177.24 ± 11.29	177.13 ± 11.24	177.4 ± 11.96	0.81
Weight [kg]	72.32 ± 15.04	71.87 ± 15.48	73 ± 15.15	0.98
Body surface [cm ²]	1.89 ± 0.26	1.88 ± 0.25	1.91 ± 0.28	0.72
BMI [kg/m ²]	22.75 ± 2.28	22.63 ± 2.54	22.92 ± 1.95	0.42

The study's protocol was approved by the appropriate Jagiellonian University Institutional Review Board (Local Bioethics Commission opinion no. 122.6120.26.2017). All enrolled study participants were provided with information on the study's objectives and gave their written informed consent before participating in the study.

Study Protocol

All studies were performed in Department of Pathophysiology, Jagiellonian University Medical College in Cracow. Autonomic nervous function was assessed based on sinus rhythm heart rate variability, systolic and diastolic blood pressure variability, and hemodynamic cardiovascular parameters. The physical examination aspect of the study protocol included ECG recording, continuous beat-to-beat blood pressure monitoring, cardio impedance measurement with HRV and BPV analysis, and determination of baroreceptor sensitivity (BRS) and parameters of cardiovascular hemodynamics using Task Force Monitor 3040i (CNSystems, Austria). The measurements were taken at rest before stimuli exposure, during both types of stimulation, and after stimuli exposure.

The measurements were taken in the afternoon (between 14.00 and 16.00) in all subjects, who were in a supine position. Study participants were asked to fast for 2 hours before the start of testing, and to avoid eating anything other than a light lunch earlier in the day or drinking fluids other than water so as to avoid any confounding effects therefrom. Study participants were also advised to have sufficient sleep the night before the study. It was ascertained that, in the 72 hours immediately preceding the study, participants were clinically stable, refrained from drinking coffee and strenuous physical exercise, and did not take any medications modulating autonomic activity.

The study took place in a specially prepared study site with comfortable ambient temperature. Every participant confirmed the comfort of their body position before the start of the experiment. Prior to the examination, study participants were provided with detailed information on the testing procedure. Outside stimuli which might have affected experimental results were reduced to a minimum throughout the duration of the study.

Determination of HRV and BPV

After a 20-min period of rest resulting in a regular and sustained respiratory rate of 14 breaths/min, ECG recordings were obtained from 6 conventional leads. All parameters were recorded for 10 minutes in the supine position during subject relaxation, for 10 minutes during exposure to the anatomy exam sound (frequency 1100 Hz, duration 4.0 sec, sound intensity 95 dB), and then for 10 minutes after exposure to the

acoustic stimulus. The staff checked the quality and consistency of the recorded data and the wellbeing of the experimental subjects before proceeding to the next acoustic stimulus exposure, all the while maintaining the minimum necessary interaction with subjects. Parameters were again recorded for 10 minutes in the supine position during relaxation, for 10 minutes during exposure to the acoustic startle stimulus (frequency 1100 Hz, 0.5 sec, 95 dB), and then for 10 minutes after stimulus exposure.

After manual editing of the obtained electrocardiograms and removal of all artifacts, the data was analyzed with Task Force Monitor V2.2 software. Frequency domain analysis of HRV and BPV was conducted. The frequency domain analysis of R-R intervals and arterial blood pressure was based on Aggregating Algorithm Regression (AAR). The time domain and nonlinear analysis of the following HRV parameters were assessed using KubiosPro 2.0 software (Kuopio, Finland): Recurrence plot analysis (%REC), Determinism (%DET), DFAa1 (short-term fractal exponent of Detrended Fluctuation Analysis that correspond to a period of 4–16 RRI) and DFAa2 (long-term fractal exponent of DFA that correspond to a period of 16–64 RRI), Sample Entropy (SampEn), and Approximate Entropy (ApEn).

The following parameters were analyzed:

Time domain HRV indices:

- SDNN — Standard deviation of all NN intervals,
- RMSSD — Square root of the mean of the sum of the squares of differences between adjacent NN intervals,
- pNN50 — Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals.

Frequency domain HRV and BPV indices:

- power spectral density (PSD) — total power of the spectrum at 0.0033–0.4 Hz,
- very low frequency (0.0033–0.04 Hz) component (VLF) — reflecting modulation by chemoreceptors of the renin — angiotensin — aldosterone system (RAAS),
- low frequency (0.04–0.15 Hz) component (LF) — reflecting modulation by the sympathetic system, associated with cyclic changes in arterial blood pressure and depending on BRS,
- high frequency (0.15–0.4 Hz) component (HF) — reflecting HRV controlled by the parasympathetic system, associated with breathing,
- LF/HF — low frequency to high frequency component ratio, a measure of the relationship between the two components of vegetative modulation,
- mid frequency (0.1 Hz) oscillations (MF), i.e. the so-called Mayer waves,
- low frequency (<0.1 Hz) oscillations (LF) — depending on many physiological phenomena. MF and LF were analyzed together as the low frequency spectrum controlled by vascular innervation and modulated by autonomic activity,
- normalized components, LFnu [LF/(TP-VLF)*100] and HFnu [HF/(TP-VLF)*100].

Nonlinear HRV analysis indices:

- Recurrence plot analysis (%REC),
- Determinism (%DET),
- DFA — the slope of the detrended fluctuation analysis estimated with a linear detrend and with a quadratic detrend, DFA α 1 (short-term fractal exponent of Detrended Fluctuation Analysis that correspond to a period of 4–16 RRI) and DFA α 2 (long-term fractal exponent of DFA that correspond to a period of 16–64 RRI),
- MSE — the slope from the multiscale entropy (MSE) measured with two different entropy estimators (the approximate entropy [ApEn] and the sample entropy [SampEn]). The entropy rate measures the increase of sequence entropy when an extra sample is added. If the entropy rate drops when the sequence will grow the process is very regular and predictable. Conversely, a constant entropy rate suggests that each new sample is not completely predictable. The entropy rate is often simplified referred to in HRV analysis as ‘entropy’,
- Poincaré plot: SD1 and SD2 is obtained by simplified plotting the values NN_{n+1} against the values of NN_n . The name stems from dynamical systems theory (a Poincaré map is a reduction of a N-dimensional continuous system to a [N – 1]-dimensional map).

Cardiovascular Hemodynamic Indices

The analyzed hemodynamic parameters included: HR — heart rate, sBP — systolic blood pressure, dBP — diastolic blood pressure, mBP — mean blood pressure (beat to beat), SV — stroke volume, SI — stroke index, CO — cardiac output, CI — cardiac index, TPR — total peripheral resistance, TPRI — total peripheral resistance index, LVET — left ventricular ejection time, ER — ejection rate, LVWI — left ventricular work index, BRS — Baroreceptor Reflex Sensitivity (spontaneous activity of baroreceptors determined using the “sequence method” which detects rising sequences, i.e. an increase in systolic blood pressure and longer R-R intervals, and falling sequences, i.e. a decrease in systolic blood pressure and shorter R-R intervals, from continuous beat-to-beat time series of R-R intervals and systolic blood pressure recordings).

Statistical Analysis

TIBCO Statistica for Windows, version 13.3 PL (TIBCO Software Inc., USA, Jagiellonian University license) was used for database management and statistical analysis. Normality of the quantitative variable distribution was verified with the Shapiro–Wilk test and equality of variances was checked with the Levene test. The statistical characteristics of quantitative variables were presented as means and standard deviations (for normally distributed data), or medians, minima, and maxima (for non-normally

distributed data). Depending on the distribution type, the unpaired T-student or Wilcoxon Rang test were used for intergroup comparisons, while the paired T-student or U Mann–Whitney test were used for intra-group comparisons. The threshold of statistical significance for all the tests was set at $p < 0.05$.

In most investigations of stress reaction, findings were based on results from recording periods ranging from 5 min to 10 min before stressor. Because we used acute acoustic stressors lasting 4 s, we did not find it necessary to analyze the 5 or 10 minutes before and after responses to the stimuli. For this reason, after careful consideration, we decided to use the 60 second period of time immediately before and after each stimulus, in keeping with the methodology of other authors investigating acute acoustic stress responses [1, 9, 17–21].

Results

As stated above, we elected to use the 60 second period of time before stimulus exposure as the baseline for estimating the influence of acute acoustic stimulus on ANS. The changes caused by exposure to the stimulus appeared a few seconds after exposure and lasted no longer than 60 seconds. We decided to use the 60 second period after stimulus exposure for analyzing SDNN, RMSSD, pNN50, SD1, SD2, SD2/SD1, ApEn, SampEn, α_1 , α_2 , the 30 second period after stimulus exposure for analyzing LF-HRV, HF-HRV, LFnu-HRV, HFnu-HRV, LF/HF ratio, HR, LVWI, TPRI, CI, ER, and the 15 second period after stimulus exposure for analyzing LF-dBPV, sBP, mBP, dBP. After these respective time intervals, the values of all indices consistently returned to baseline values. Observations from selected parameters are presented in Figure 1.

In the resting period, the analysis of frequency domain HRV (LF, HF, LFnu, HFnu, LF/HF ratio), time domain HRV (SDNN, RMSSD, pNN50, nonlinear HRV), Poincaré plot (SD1, SD2, SD2/SD1, ApEn, SampEn, α_1 , α_2), blood pressure variability (LF-dBPV), and selected cardiovascular hemodynamic parameters (HR, sBP, mBP, dBP, LVWI, TPRI, CI, ER) did not show differences between the medical student group and the non-medical student group.

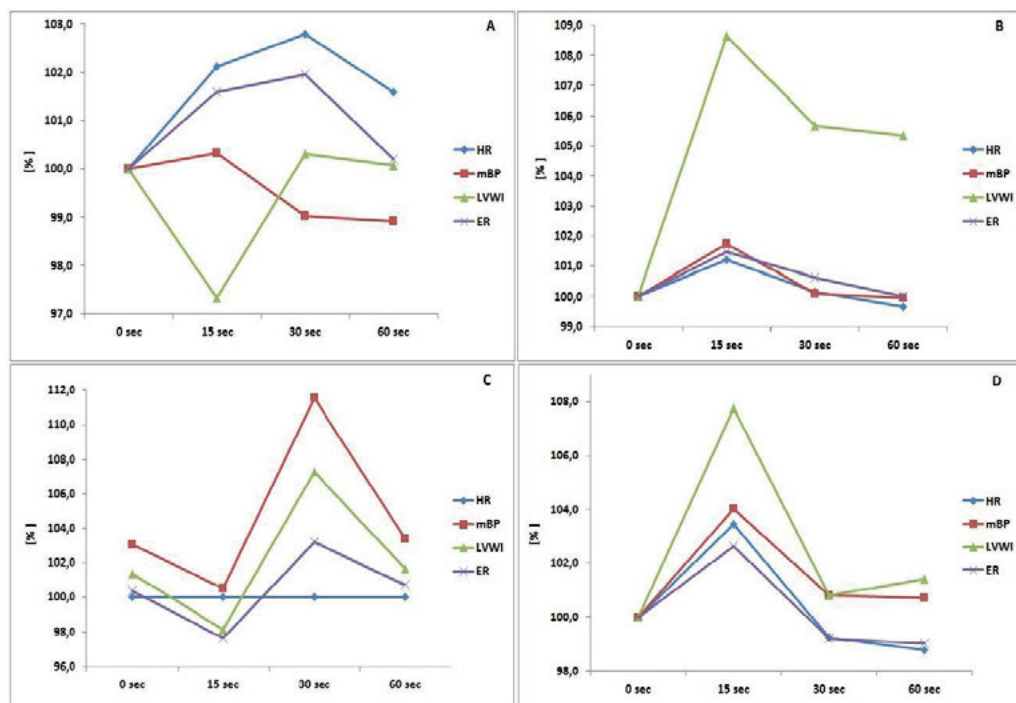


Fig. 1. The percentage changes of selected cardiovascular parameters in response to the stress stimuli in medical and non-medical groups. The maximal effect after both stressors activation were in medical group students in 30 seconds (A, C), but in non-medical students were early in 15 second (B, D). Legend: A. Medical — the “pins” exam sound, B. Non-medical “pins” exam sound, C. Medical — acoustic startle, D. Non-medical — acoustic startle. HR — heart rate, mBP — mean blood pressure, LVWI — left ventricular work index, ER — ejection rate.

Linear and Nonlinear HRV analysis

The analysis of autonomic nervous system indicators shows differences in response between the medical student and non-medical student groups. All statistically significant results revealed that in the medical student group, the reaction to the anatomy exam sound is markedly increased than the response to the acoustic startle sound. Further, a greater number of indicators demonstrated a stronger response to the acoustic startle sound than the anatomy exam sound (LF, LFnu, dBP) in the non-medical student group (Table 5).

Medical student group

Every kind of stress stimulation (acoustic startle; “pins” sound) caused changed values of HRV indicators, but only after the “pins” sound was it significant (LFnu 52.09% vs. 55.39%; $p = 0.082$, HFnu 47.91% vs. 44.61%; $p = 0.048$). The increase in LF after the “pins” sound was also significantly higher than after the acoustic startle (LF 782.34 ms^2 vs. 1018.78 ms^2 , $p = 0.033$) (Table 2).

Table 2. The changes of time and frequency domain analysis parameters of HRV in medical and non-medical students' groups in response to the stress's stimulus.

HRV	Groups	“pins” exam sound		acoustic startle		<i>p</i>
		Pre stimuli	Post stimuli	Pre stimuli	Post stimuli	
SDNN [ms]	Medical	57.25	68.33	60.43	80.31	0.237/ 0.0045[#]
	Non-medical	65.65	84.77	73.09	80.96	0.443/ 0.037[*]
	<i>p</i>	0.523	0.560	0.081	0.598	
RMSSD [%]	Medical	50.78	52.63	53.1	60.99	0.061/ 0.019[#]
	Non-medical	68.64	82.94	64.02	74.44	0.421/ 0.016[*]
	<i>p</i>	0.1852	0.068	0.355	0.292	
pNN50 [%]	Medical	28.18	28.06	29.16	34.65	0.157
	Non-medical	41.16	44	37.03	40.29	0.593
	<i>p</i>	0.209	0.131	0.348	0.486	
LFnu [%]	Medical	52.09	55.39	49.38	48.51	0.082 ^{&}
	Non-medical	50.08	49.11	56.21	60.12	0.087 ^{&}
	<i>p</i>	0.769	0.373	0.292	0.041	
HFnu [%]	Medical	47.91	44.61	50.61	51.5	0.048^{&}
	Non-medical	49.92	50.89	43.79	39.88	0.087 ^{&}
	<i>p</i>	0.769	0.373	0.292	0.041	
LF [ms^2]	Medical	782.34	1018.78	900.38	1002.86	0.032[*]
	Non-medical	1033.2	1030.06	1255	1478.88	0.036^{&}
	<i>p</i>	0.259	0.872	0.099	0.186	
HF [ms^2]	Medical	865.19	875.94	948.94	1081.05	0.078/ 0.047[#]
	Non-medical	719.05	787.97	823.75	967.8	0.327
	<i>p</i>	0.923	0.923	0.675	0.974	

Legend: SDNN — standard deviation of normal to normal (beats), RMSSD — root mean square of successive differences, pNN50 — the number of pairs that differ by more than 50 ms as a percentage of all numbers of normal beats, LF — low frequency component (0.04–0.15 Hz), HF — frequency component (0.15–0.4 Hz), LFnu — low frequency normalized units, HFnu — high frequency normalized units, LF/HF ratio-low frequency component to high frequency component ratio. * — statistical significant between before and in response to “pins” exam sound; # — statistical significant between before and in response to acoustic startle; & — statistical significant between response to “pins” exam sound and acoustic startle.

The time domain HRV analysis parameters (SDNN, RMSSD) increased after acoustic stress, but pNN50 remain unchanged after both the acoustic startle and the “pins” sound.

Nonlinear analysis of HRV indicated significantly increased ApEn ($p = 0.041$) and Shannon Entropy ($p = 0.012$) in response to “pins” sound. Acoustic startle stimulus caused an increase of Poincaré plot SD1 (0.019) and SD2 ($p = 0.008$). The exact values of these indices are shown in Table 3.

Table 3. The changes of nonlinear analysis parameters of HRV in response to the stress stimulus in medical and non-medical students’ groups.

Nonlinear HRV analysis	Groups	“pins” exam sound		acoustic startle		<i>p</i>
		Pre stimuli	Post stimuli	Pre stimuli	Post stimuli	
SD1	Medical	36.18	37.49	37.86	43.47	<i>0.086/0.019[#]</i>
	Non-medical	48.92	59.09	46.61	53.02	<i>0.419/0.016*</i>
	<i>p</i>	0.185	0.068	0.358	0.295	
SD2	Medical	71.53	87.62	75.65	103.25	<i>0.286/0.008[#]</i>
	Non-medical	78.09	103.64	91.63	100.33	0.291
	<i>p</i>	0.616	0.328	0.064	0.873	
SD2/SD1	Medical	2.19	2.82	2.16	2.54	0.156
	Non-medical	1.83	1.95	2.37	2.08	0.646
	<i>p</i>	0.331	0.043	0.677	0.166	
ApEn	Medical	0.46	0.55	0.5	0.48	0.041*
	Non-medical	0.51	0.46	0.51	0.49	0.235
	<i>p</i>	0.112	0.061	0.773	0.636	
SampEn	Medical	1.51	1.38	0.175	1.59	0.078 ^{&}
	Non-medical	1.97	1.74	1.47	1.94	<i>0.575/0.066^{&}</i>
	<i>p</i>	0.033	0.101	0.257	0.212	
Recurrency	Medical	26.4	30.65	27.33	29.84	0.845
	Non-medical	26.33	21.34	28.79	31.7	0.036^{&}
	<i>p</i>	0.985	0.093	0.719	0.669	
Determision	Medical	96.23	97.15	97.16	97.46	0.513
	Non-medical	96.49	96.05	97.07	97.58	0.047^{&}
	<i>p</i>	0.721	0.169	0.897	0.864	
Shannon Entropy	Medical	2.61	2.75	2.67	2.74	0.012
	Non-medical	2.56	2.47	2.7	2.89	<i>0.382/0.005^{&}</i>
	<i>p</i>	0.656	0.058	0.772	0.243	

Legend: ApEn — approximate entropy, SampEn — sample entropy, SD — standard deviation. * — statistical significant between before and in response to “pins” exam sound; # — statistical significant between before and in response to acoustic startle; & — statistical significant between response to “pins” exam sound and acoustic startle.

Non-medical student group

The HRV parameters were higher after the acoustic startle stimulus than after the “pins” sound in this group (LFnu 60.12 % vs. 49.11%; $p = 0.087$, LF 1478.88 ms² vs. 1030.06 ms², $p = 0.036$). The HFnu parameter was also changed after the acoustic startle, being lower (HFnu 39.88% vs 50.89 %; $p = 0.087$) than after the “pins” sound (Table 2).

The time domain HRV analysis parameters (SDNN, RMSSD) increased after the “pins” sound, but pNN50 remain unchanged after both the acoustic startle and the “pins” sound.

Nonlinear analysis of HRV indicated a significant increase of ApEn ($p = 0.041$) and Shannon Entropy ($p = 0.012$) in response to the “pins” sound stimulus. The acoustic startle stimulus caused an increase of the Poincaré plot SD1 (0.019) and SD2 ($p = 0.008$). The exact values of these indices are shown in Table 3.

BPV Analysis

The LF-dBP parameter of diastolic BPV was significantly higher in response to the “pins” sound stimulation (5.92 ms² vs. 6.19 ms²; $p = 0.039$) in the medical student group than in the non-medical student group. The response to the acoustic startle stimulus when compared with the “pins” sound stimulus was significantly lower in both investigated groups ($p = 0.046$ vs. $p = 0.022$). The exact values of the BPV parameters are shown in Table 4.

Table 4. The changes of cardiovascular hemodynamic parameters in response to the stress stimulus in medical- and non-medical students’ groups.

Hemody- namic para- meters	Groups	“pins” exam sound		acoustic startle		<i>p</i>
		Pre stimuli	Post stimuli	Pre stimuli	Post stimuli	
HR [beats/min]	Medical	72	74	69	70	0.004^{&}
	Non-medical	68	69	68	68	0.695
	<i>p</i>	0.067	0.051	0.395	0.517	
sBP [mmHg]	Medical	110.06	109.34	107.77	106.80	0.555
	Non-medical	112.29	115.13	111.47	113.64	0.700
	<i>p</i>	0.509	0.296	0.664	0.398	
dBP [mmHg]	Medical	65.46	66.67	68.96	70.23	0.315
	Non-medical	67.24	68.52	68.11	71.46	0.466/ 0.038[#]
	<i>p</i>	0.699	0.728	0.805	0.778	
mBP [mmHg]	Medical	82.72	82.99	85.71	86.15	0.408
	Non-medical	84.87	86.34	85.15	88.58	0.56/ 0.037[#]
	<i>p</i>	0.652	0.542	0.888	0.642	

CI [l/(min*m)]	Medical	3.76	3.80	3.62	3.71	0.266
	Non-medical	3.47	3.56	3.16	3.16	0.159
	<i>p</i>	0.792	0.619	0.306	0.120	
ER [%]	Medical	35.68	36.38	34.03	34.60	0.003^{&}
	Non-medical	34.79	35.01	34.02	33.75	0.253
	<i>p</i>	0.846	0.560	0.677	0.718	
TPRI [dyne*s*m _c / cm ⁵]	Medical	1870.2	1759.88	1954.91	1889.31	0.259/ 0.041[*]
	Non-medical	2079.31	2034.88	2290.69	2313.12	0.268
	<i>p</i>	0.479	0.324	0.231	0.179	
LVWI [mmHg*l/ min/m _c]	Medical	4.08	4.1	4.1	4.07	0.932
	Non-medical	3.92	4.14	3.57	3.6	0.153/ 0.012[*]
	<i>p</i>	0.79	0.935	0.244	0.285	
LF-dBP	Medical	5.92	6.19	4.1	4.04	0.039/0.046^{&}
	Non-medical	5.94	6.02	4.06	4	0.022^{&}
	<i>p</i>	0.637	0.637	0.978	0.978	
BRS [ms/mmHg]	Medical	25.16	25.25	25.91	25.82	0.334
	Non-medical	34.9	34.77	30.87	30.72	0.445/0.07 [#]
	<i>p</i>	0.212	0.174	0.192	0.233	

Legend: HR — heart rate, sBP — systolic blood pressure, mBP — medium blood pressure, dBP — diastolic blood pressure, LF-dBP — low frequency diastolic blood pressure, LVWI — left ventricular work index, TPRI — total peripheral resistance index, CI — cardiac index, ER- ejection rate. * — statistical significant between before and in response to “pins” exam sound; # — statistical significant between before and in response to acoustic startle; & — statistical significant between response to “pins” exam sound and acoustic startle.

Cardiovascular Hemodynamic Parameters Analysis

Heart rate was significantly higher in response to the “pins” sound stimulus (74 beats/min) than the acoustic startle stimulus (70 beats/min) in the medical student group ($p = 0.004$), but there were no differences in heart rate observed between the two stimuli in the non-medical student group.

While significant differences in systolic BP and CI were not observed in either investigated group, significant increases after exposure to the acoustic startle stimulus were observed in the dBP and mBP of the non-medical student group (dBP 68.11 mmHg vs. 71.48 mmHg, $p = 0.038$; mBP 85.15 mmHg vs. 88.58 mmHg, $p = 0.037$). ER was significantly higher in the medical student group in response to the “pins” sound stimulus than the acoustic startle stimulus (36.38% vs. 34.60%; $p = 0.003$). TPRI was significantly decreased after the “pins” sound stimulus than the acoustic startle stimulus. Analysis of individual recordings of hemodynamic parameters showed that the more sustained response to both stress stimuli was in the

medical student group (about 30 seconds vs. 15 seconds for the non-medical student group). The results are shown in Table 4, Figure 1.

Baroreceptor Activity

In response to the stress stimuli, we did not observe significant differences in baroreceptor sensitivity in the medical student group, but the non-medical student group showed a non-significant decrease of BRS in response to the “pins” sound stimulus and the acoustic startle stimulus ($p = 0.07$, Table 4).

Stress Questionnaire

Analysis of the responses from the stress questionnaire showed that the investigated groups have a similar level of coping with stress: 13% perform well under stress in the medical group, 10% in non-medical group; good — 53% vs. 60% respectively; poorly — 33.2% vs. 30% respectively. Likewise, the comfort of living with current levels of stress was similar (73% vs. 80%). A significant difference was found in the case of work (science) under stress — 73.3% of those questioned in the medical student group found good coping mechanisms in this regard, but in the non-medical student group only 40% responded positively ($p = 0.02$).

Discussion

The main goal of this study was to demonstrate a difference in response to two different sound stressor stimuli (the acoustic startle stimulus vs. the “pins” sound stimulus) in a group of healthy volunteers. Effects on autonomic nervous system activity were determined based on linear and nonlinear analysis of HRV, the frequency domain analysis of BPV, baroreceptors sensitivity, and correlation with cardiovascular system response (hemodynamic parameters) by stress stimulation. An extensive literature review found that this is the first study to analyse the relationship and differences between acoustic stressors which can activate the ANS in different student groups (medical students and non-medical students). The principal findings of our study can be summarized as follows:

- 1) The value of parameters from linear and nonlinear HRV analysis and cardiovascular recordings at rest were similar in both groups, medical and non-medical.
- 2) All sound stimuli caused autonomic nervous system responses in participants; however, parameters indicating stimulation from the ANS were significantly higher in the medical student group due to the anatomy exam “pins” acoustic stimulus.
- 3) In the non-medical student group, indicators of ANS activity and hemodynamic parameters revealed a stronger response to the acoustic startle stimulus.

Feluś *et al.* [18] provoked sympathetic activation of the ANS in healthy volunteers and celiac patients in response to a sound stressor. There were the significant increases in LFnu and decreases in HFnu parameters of HRV. The response depended on the resting activity of the sympathetic ANS, with excessive activity causing a diminished response to the stimulus. Cheng *et al.* [5] were noted a decrease in the HF component of HRV in response to exposure to loud metal music. Similar changes were observed in our study. These observations showed that different types of sound stimuli may produce similar results. In our study, sound stimuli were short (4 s or 0.5 s), as opposed to the Cheng *et al.* study, where the sound stimuli lasted for minutes.

Walker *et al.* [6] demonstrated that sound stimuli lead to a decline in SDNN. In our study, we observed the opposite effect, but changes of SDNN, pNN50 and RMMSD were non-significant. These discrepancies between studies could be caused by differences in the frequency of sounds. As our study utilized high frequency sound stimuli, this could explain the loss of SDNN decline in our investigation. As in our study, Walker *et al.* observed an increase in both systolic and diastolic blood pressure after exposure to the stressor [6].

The novelty of our investigation was that we not only evaluated differences in response to the stressors in time and frequency domain HRV analysis, but we also used parameters of nonlinear HRV analysis. We demonstrated that ApEn increased due to the “pins” acoustic stimulus in the medical student group, but also that in non-medical student group, the Shannon entropy increased in response to the acoustic startle stimulus. This indicates a specific response to different stressors dependent on the particular group being investigated. According to Buccelletti *et al.* [11], Approximate Entropy [ApEn] is a measure of the degree of heartbeat irregularity, with greater values of ApEn meaning less heartbeat regularity. We noticed higher increases of ApEn in the medical student group after exposure to the “pins” sound stimulus than after exposure to the acoustic startle stimulus. Reaction to the acoustic startle stimulus was similar in both groups, with ApEn in the medical student group and the non-medical student group 0.5 vs. 0.51, respectively, before stimulus exposure and 0.48 vs. 0.49, respectively, after stimulus exposure, which confirmed a specific response in the medical student group. According to Carrillo *et al.* [22], elevation of SD1 and SD2 is connected with activation of the parasympathetic nervous system. The non-medical student group had a statistically non-significant greater level of SD 1 and SD2 from Poincaré plot, which may suggest that the cardiac vagal modulation of medical students is worse than that of other non-medical students of the same age [22].

The heart rate response to the “pins” sound stimulus was significantly higher than to the acoustic startle stimulus in the medical student group, but the non-medical student group showed significant increases in dBp and mBP after exposure to the acoustic startle stimulus. Changes in cardiovascular hemodynamic parameters did not

confirm the analysis of the BRS. Absence of significant differences in baroreceptors sensitivity is probably explained by the short period of exposure to the acoustic stimuli. Additionally, analysis of temporal changes in the subjects' cardiovascular parameters showed that the highest response to both acoustic stimuli occurred 15 to 30 seconds after exposure to the acoustic stimulus. Our study partially confirmed Cheng *et al.* [5], where an increase in heart rate and blood pressure was demonstrated in the study subjects in response to loud metal music exposure. This overactivity of the sympathetic component of the ANS occurred for about 20 seconds after exposure to the stimulus, similarly to what was demonstrated in our data. Maximal sympathetic activation occurred within 30 seconds post-exposure in the medical student group, whereas it occurred within 15 seconds in the non-medical student group.

Differences between the groups could be explained by "resistance" to stress in the medical student group, whose study program and requirements tempered them to work under pressure. Adlan *et al.* [14] indicated another probable mechanism: an acute release of cortisol causing an increase in HR and BP, with concurrent reduction of cardiovagal baroreflex sensitivity and heart rate variability in young men. Our study had some limitations, among them the small group sizes of medical students and non-medical students, as well as differences in athletic activity in both groups. Likewise, the memories linked with the anatomy exam may not always be recognized as stressful ones in certain groups of medical students. Our results showed that not only current mental stress can stimulate the ANS, especially such as is experienced during an exam, but even factors like a sound stimulus connected with previously stressful events, such as the signal used in a past practical anatomy exam, can achieve similar ANS stimulation. Our results indicate the necessity of further investigation to determine whether there exist differences in such ANS responses dependent on the duration of time since the exam or dependent on gender differences [23, 24].

In conclusion, our research confirmed the hypothesis that the acoustic stimulus associated with stressful memories caused a greater response of the autonomic nervous system in the medical student group, which correlated with hemodynamic indicators of the circulatory system.

A new finding of our study was that even very short stressful acoustic stimuli can cause the stimulation of the sympathetic ANS component. This excessive activity can persist for up to about 1 minute. Therefore, repeated exposure to such stimuli every day, multiple times a day, can be a significant risk factor for the development of cardiovascular diseases.

Conclusion

Both acoustic stimuli activated the sympathetic ANS. The anatomy practical exam sound, which medical students remembered from the past, more strongly stimulated

the sympathetic autonomic nervous system in the medical student group than in the non-medical student group.

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Conflict of interest

None declared.

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